ATTACHMENT A

Clean version of the claims

1. A process for obtaining yeast strains conserving stress resistance in the presence of fermentable sugars, comprising the following steps:

a mutagenic treatment is carried out on the cells of a starting strain,

the cells having undergone the said mutagenic treatment are cultured so as to obtain a stationary phase,

the said cells in stationary phase are incubated in the presence of at least one fermentable sugar selected from the group comprising glucose, maltose, and sucrose, this sugar being present in a quantity such that the cells enter an active metabolic state (fermentation and/or growth),

said cells in active metabolic state are subjected to one or several stresses leading to a mortality rate of at least 99% with respect to the starting population,

the surviving cells are isolated and

those of the surviving cells which respond to the following criteria which characterize the fil phenotype are selected

- a growth, evaluated by production or production yield of biomass over sugar in a given time or by a growth rate, under identical culture conditions, at least equal to 80% of the starting strain,
- a CO_2 release, or a metabolite production, in identical conditions, at least equal to 80% of the starting strain,
- a stress resistance, corresponding to a survival rate at least 2 times higher than the survival rate of the starting strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 1.5 times higher than the survival rate of the starting strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at -20°C or at a lower temperature,
- maintenance of these properties after repeated cultures on non selective medium, so as to verify that the fil phenotype obtained by the mutation is perfectly stable and permanent.

2. A process according to claim 1, wherein it is checked that the selected yeast strains present an alcohol assimilation, under identical conditions, at least equal to 50% of that of the starting strain and that the selected yeast strains do not produce metabolites which give a bad smell or a bad or abnormal taste to breads.

3. A process according to claim 1, wherein the starting strain is an

industrial strain.

4. A process according to claim 3, wherein an industrial fil mutant carrying several mutations is obtained and wherein:

- the segregants issued from this industrial mutant are crossed with a laboratory haploid strain to select the segregant issued from this industrial mutant giving to the polyploids obtained with the laboratory strain an improvement in the required properties;
- the segregants thus selected are crossed one with the other;
- the polyploids obtained are selected according to the criteria of fil phenotypes defined in claim 1.

5. A process according to claim 1, wherein the selected fil strains have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 50% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

6. A process according to claim 1, wherein the cells obtained after mutagenic treatment are introduced into pieces of dough subjected to at least 100 cycles of freezing/thawing after a first fermentation of the dough of 30 minutes at 30°C.

7. (twice amended) An industrial yeast strain of the fil phenotype having a survival rate, in growth phase on glucose, of at least 50% after heat treatment, the growth phase being defined as a cultivation of stationary cells on glucose for 10 minutes at 30°C after stationary phase.

9. A strain according to claim 7, belonging to *Saccharomyces cerevisiae* species.

10. A yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 50% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on glucose of 10 minutes at 30°C after stationary phase.

12. An industrial yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of $(NH_4)_2S0_4$ and 160mg of dry matter of the considered strain, defined by the ratio between the release of CO_2 at 30°C after 1 month or 30 days of conservation at -20°C and the release of CO_2 at 30°C after 1 day of conservation at -20°C, is at least equal to 80%.

14. A yeast strain according to claim *57*, whose loss of released gas after drying of the biomass harvested in a phase close to exponential growth phase is at most equal to 67% of the

loss of released gas after drying of yeasts obtained using the corresponding starting strain.

15. (twice amended) Strain PVD115O = M5 *fill* deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.) under the n° I-2031 and the n° I-2203.

16. Strain KL1 = W303 *fi12* deposited at C.N.C.M. under the n° I-2032.

17. Strain FD51 = HL816 *fi1300* deposited at C.N.C.M. under the n° I-2033.

18. Strain FDH16-22 = HL822 *fil300* deposited at C.N.C.M. under the n° I-2034.

19. Strain AT25 = S47 *fi1400* deposited at C.N.C.M. under the n° I-2035.

20. Strain AT28 = S47 *fil500* deposited at C.N.C.M. under the n° I-2036.

21. Strain AT251 deposited at C.N.C.M. under the n° I-2222.

22. Strain AT252 deposited at C.N.C.M. under the n° I-2223.

23. Strain AT254 deposited at C.N.C.M. under the n° I-2224.

38. A dry baker's yeast obtained by culturing a strain according to claim 7.

40. A brewery yeast obtained by culturing a strain according to claim 7.

41. A yeast intended for the production of alcohol obtained by culturing a strain according to claim 7.

42. A process according to claim 1, wherein the yeast strains are of the Saccharomyces cerevisiae species.

43. A process according to claim 1, wherein the selected yeast strains present a growth, evaluated by production or production yield of biomass over sugar in a given time or by a growth rate, under identical culture conditions, at least equal to 90% of the starting strain.

44. A process according to claim 1, wherein the selected yeast strains present a CO_2 release, or a metabolite production, in identical conditions, at least equal to 90% of the starting strain.

45. A process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least 3 times higher than the survival rate of the starting strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 2 times higher than the survival rate of the starting strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at -20°C or at a lower temperature.

46. A process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least *5* times higher than the survival rate of the starting strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 3 times higher than the

survival rate of the starting strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at -20°C or at a lower temperature.

47. A process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least 10 times higher than the survival rate of the starting strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least *5* times higher than the survival rate of the starting strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at 20°C or at a lower temperature.

48. A process according to claim 1, wherein the selected fil strains have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 60% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

49. A process according to claim 1, wherein the selected fil strains have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 70% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

50. A process according to claim 1, wherein the selected fil strains have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 80% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

51. An industrial yeast strain according to claim 7 belonging to the Saccharomyces genus.

52 A yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 60% after a heat treatment of 20 minutes at *52°C,* the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary phase.

53. A yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 70% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary phase.

54. A yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least *75%* after a heat treatment of 20 minutes at *52°C,* the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary

phase.

55. An industrial yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of $(NH_4)_2SO_4$ and 160mg of dry matter of the considered strain, defined by the ratio between the release of CO_2 at 30°C after 1 month or 30 days of conservation at -20°C and the release of CO_2 at 30°C after 1 day of conservation at -20°C, is at least equal to 85%.

56. An industrial yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of $(NH_4)_2S0_4$ and 160mg of dry matter of the considered strain, defined by the ratio between the release of CO_2 at 30°C after 1 month or 30 days of conservation at -20°C and the release of CO_2 at 30°C after 1 day of conservation at -20°C, is at least equal to 90%.

57. An industrial yeast strain having the fil phenotype, obtainable by the process according to claim 1, presenting an alcohol assimilation, under identical conditions, at least equal to 50% of that of the starting strain and not producing metabolites which give a bad smell or a bad or abnormal taste to breads.

58. A yeast strain according to claim 57, whose loss of released gas after drying of the biomass harvested in a phase close to exponential growth phase is at most equal to 50% of the loss of released gas after drying of yeasts obtained using the corresponding starting strain.

59. A baker's yeast obtained by culturing a yeast strain according to claim 7.

60. (new) An industrial yeast strain of the fil phenotype presenting a stability to freezing in pieces of dough containing 20g of flour, 15g of water, 1g of sucrose, 0.405g of NaCl, 0.06g of $(NH_4)_2SO_4$ and an amount of the industrial yeast corresponding to 160mg of yeast dry matter, higher than 60%, said stability being defined as the ratio between the release of CO_2 at 30°C after 30 days of conservation at -20°C and the release of CO_2 at 30°C after 1 day of conservation at -20°C, whereby before freezing at -20°C, the pieces of dough are incubated at 30°C for 30 minutes.

61. (new) An industrial yeast strain of the fil phenotype presenting a stability to freezing in pieces of dough containing 20g of flour, 15g of water, 1g of sucrose, 0.405g of NaCl, 0.06g of $(NH_4)_2SO_4$ and an amount of the industrial yeast corresponding to 160mg of yeast dry matter, higher than 80%, said stability being defined as the ratio between the release of CO_2 at 30°C after 30 days of conservation at -20°C and the release of CO_2 at 30°C after 1 day of conservation

at -20°C, whereby before freezing at -20°C, the pieces of dough are incubated at 30°C for 30 minutes.